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Lisa M. Nash

Date: August 25, 2003

PROVISIONAL APPLICATION COVER SHEET

This is a request for a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Docket No. 20662.002	Type a plus sign (+) inside this box → +

INVENTOR(S)/APPLICANT(S)			
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TITLE OF INVENTION (280 characters max)

Immunogenic Compositions for *Chlamydia trachomatis*

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ENCLOSED APPLICATION PARTS (check all that apply)

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_X_ A check or money order is enclosed to cover the Provisional filing fees

_X_ The Commissioner is hereby authorized to charge any additional fees and credit Deposit Account Number 03-1664.	PROVISIONAL FILING FEE AMOUNT ENCLOSED **$160.00** CHECK NO. 8202

The invention was made by an agency of the United States Government or under a contract with an agency of the United States government.

_X_ No

___ Yes, the name of the U.S. Government agency and the Government contract number are:

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IMMUNOGENIC COMPOSITIONS FOR *CHLAMYDIA TRACHOMATIS*

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the fields of immunology and vaccinology. In particular, it relates to antigens derived from *Chlamydia trachomatis* and their use in immunisation.

BACKGROUND ART

The *Chlamydiae* are obligate intracellular parasites of eukaryotic cells which are responsible for endemic sexually transmitted infections and various other disease syndromes. They occupy an exclusive eubacterial phylogenic branch, having no close relationship to any other known organisms.

Historically, the *Clamydiae* have been classified in their own order (*Chlamydiales*) made up of a single family (*Chlamydiaceae*) which in turn contains a single genus (*Chlamydia*, also referred to as *Chlamydophila*). More recently, this order has been divided into at least four families including *Chlamydiaceae, Parachlamydiaceae, Waddiaceae* and *Simkaniaceae.* In this more recent classification, the *Chlamydiaceae* family includes genuses of *Chlamydophila* and *Chlamydia*, *Chlamydia trachomatis* being a species within the *Chlamydia* genus. See Ref. 1.

A particular characteristic of the *Chlamydiae* is their unique life cycle, in which the bacterium alternates between two morphologically distinct forms: an extracellular infective form (elementary bodies, EB) and an intracellular non-infective form (reticulate bodies, RB). The life cycle is completed with the re-organization of RB into EB, which leave the disrupted host cell ready to infect further cells.

The genome sequences of at least five chlamydia or chlamydophila species are currently known – *C.trachomatis, C.pneumoniae, C.muridarum, C.pecorum* and *C.psittaci* (See Refs. 2, 8).

The human serovariants ("serovars") of *C.trachomatis* are divided into two biovariants ("biovars"). Serovars A-K elicit epithelial infections primarily in the ocular tissue (A-C) or urogenital tract (D-K). Serovars L1, L2 and L3 are the agents of invasive lymphogranuloma venereum (LGV).

Although chlamydial infection itself causes disease, it is thought that the severity of symptoms in some patients is actually due to an aberrant host immune response. Failure to clear the infection results in persistent immune stimulation and, rather than helping the host, this results in chronic infection with severe consequences, including sterility and blindness. See, e.g., Ref. 9. In addition, the protection conferred by natural chlamydial infection is usually incomplete, transient, and strain-specific.

Due to the serious nature of the disease, there is a desire to provide suitable vaccines. These may be useful (a) for immunisation against chlamydial infection or against chlamydia-induced disease (prophylactic vaccination) or (b) for the eradication of an established chronic chlamydial infection (therapeutic vaccination). Being an intracellular parasite, however, the bacterium can generally evade antibody-mediated immune responses.

Various antigenic proteins have been described for *C.trachomatis*, and the cell surface in particular has been the target of detailed research. See, *e.g.*, Ref. 10. These include, for instance, Pgp3 (Refs. 11, 12, and 13), MOMP (Ref. 14), Hsp60 (GroEL) (Ref. 15) and Hsp70 (DnaK-like) (Ref. 16). Not all of these have proved to be effective vaccines, however, and further candidates have been identified. See Ref. 17.

Vaccines against pathogens such as hepatitis B virus, diphtheria and tetanus typically contain a single protein antigen (*e.g.* the HBV surface antigen, or a tetanus toxoid). In contrast, acellular whooping cough vaccines typically have at least three *B.pertussis* proteins, and the Prevnar™ pneumococcal vaccine contains seven separate conjugated saccharide antigens. Other vaccines such as cellular pertussis vaccines, the measles vaccine, the inactivated polio vaccine (IPV) and meningococcal OMV vaccines are by their very nature complex mixtures of a large number of antigens. Whether protection can be elicited by a single antigen, a small number of defined antigens, or a complex mixture of undefined antigens, therefore depends on the pathogen in question.

It is an object of the invention to provide further and improved compositions for providing immunity against chlamydial disease and/or infection. The compositions are based on a combination of two or more (*e.g.* three or more) *C.trachomatis* antigens.

DISCLOSURE OF THE INVENTION

Within the ~900 proteins described for the *C.trachomatis* genome of reference 5, Applicants have discovered a group of five *Chlamydia trachomatis* antigens that are particularly suitable for immunisation purposes, particularly when used in combinations. The invention therefore provides a composition comprising a combination of *Chlamydia trachomatis* antigens, said combination consisting of two, three, four or all five *Chlamydia trachomatis* antigens of a first antigen group, said first antigen group consisting of: (1) PepA; (2) LcrE; (3) ArtJ; (4) DnaK; and (5) CT398. These antigens are referred to herein as the 'first antigen group'.

Preferably, the composition of the invention comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of: (1) PepA & LcrE; (2) PepA & ArtJ; (3) PepA & DnaK; (4) PepA & CT398; (5) LcrE & ArtJ; (6) LcrE & DnaK; (7) LcrE & CT398; (8) ArtJ & DnaK; (9) ArtJ & CT398; (10) DnaK & CT398; (11) PepA, LcrE & ArtJ; (12) PepA, LcrE & DnaK; (13) PepA, LcrE & CT398; (14) PepA, ArtJ & DnaK; (15) PepA, ArtJ and CT398;

(16) PepA, DnaK & CT398; (17) LcrE, ArtJ & DnaK; (18) LcrE, ArtJ & CT398; (19) LcrE, DnaK & CT398; (20) ArtJ, DnaK & CT398; (21) PepA, LcrE, ArtJ & DnaK; (22) PepA, LcrE, DnaK & CT398; (23) PepA, ArtJ, DnaK & CT398; (24) PepA, LcrE, ArtJ & CT398; (25) LcrE, ArtJ, DnaK & CT398; and (26) PepA, LcrE, ArtJ, DnaK & CT398. Preferably, the composition of *Chlamydia trachomatis* antigens consists of PepA, LcrE, ArtJ, DnaK & CT398.

The invention also provides for a slightly larger group of 13 *Chlamydia trachomatis* antigens that are particularly suitable for immunisation purposes, particularly when used in combinations. (This second antigen group includes the five *Chlamydia trachomatis* antigens of the first antigen group.) These 13 *Chlamydia trachomatis* antigens form a second antigen group of (1) PepA; (2) LcrE; (3) ArtJ; (4) DnaK; (5) CT398; (6) OmpH-like; (7) L7/L12; (8) OmcA; (9) AtoS; (10) CT547; (11) Eno; (12) HtrA and (13) MurG. These antigens are referred to herein as the 'second antigen group'.

The invention therefore provides a composition comprising a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen *Chlamydia trachomatis* antigens of the second antigen group. Preferably, the combination is selected from the group consisting of two, three, four or five *Chlamydia trachomatis* antigens of the second antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens of the second antigen group.

Each of the *Chlamydia trachomatis* antigens of the first and second antigen group are described in more detail below.

(1) PepA leucyl aminopeptidase A protein

One example of a 'PepA' protein is disclosed as SEQ ID NO[s]: 71 & 72 in reference 17 {GenBank accession number: AAC67636, GI:3328437; 'CT045'; SEQ ID NO: 2 below}. It is believed to catalyse the removal of unsubstituted N-terminal amino acids from various polypeptides.

Preferred PepA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 2; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 2, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PepA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 2. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 2. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 2. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

The PepA protein may contain manganese ions.

SEQ ID NO: 2

MVLLYSQASWDKRSKADALVLPFWMKNSKAQEAAVVDEDYKLVYQNALSNFSGKKGETAFLFGNDHTKEQKIVLLGLGKSEEVSGTTV
LEAYAQATTVLRKAKCKTVNILLPTISQLRFSVEEFLTNLAAGVLSLNYNYPTYHKVDTSLPFLEKVTVMGIVSKVGDKIFRKEESLF
EGVYLTRDLVNTNADEVTPEKLAAVAKDLAGEFASLDVKILDRKAILKEKMGLLAAVAKGAAVEPRFIVLDYQGKPKSKDRTVLIGKG
VTFDSGGLDLKPGKAMITMKEDMAGAATVLGIFSALASLELPINVTGIIPATENAIGSAAYKMGDVYVGMTGLSVEIGSTDAEGRLIL
ADAISYALKYCNPTRIIDFATLTGAMVVSLGESVAGFFANNDVLARDLAEASSETGEALWRMPLVEKYDQALHSDIADMKNIGSNRAG
SITAALFLQRFLEDNPVAWAHLDIAGTAYHEKEELPYPKYATGFGVRCLIHYMEKFLSK

(2) LcrE low calcium response E protein

One example of a 'LcrE' protein is disclosed as SEQ ID NO[s]: 61 & 62 in reference 17 {GenBank accession number: AAC67680, GI:3328485; 'CT089'; SEQ ID NO: 3 below}.

Preferred LcrE proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 3; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 3, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These LcrE proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 3. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 3. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 3. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 3

MTASGGAGGLGSTQTVDVARAQAAAATQDAQEVIGSQEASEASMLKGCEDLINPAAATRIKKKGEKFESLEARRKPTADKAEKKSEST
EEKGDTPLEDRFTEDLSEVSGEDFRGLKNSFDDDSSPDEILDALTSKFSDPTIKDLALDYLIQTAPSDGKLKSTLIQAKHQLMSQNPQ
AIVGGRNVLLASETFASRANTSPSSLRSLYFQVTSSPSNCANLHQMLASYLPSEKTAVMEFLVNGMVADLKSEGPSIPPAKLQVYMTE
LSNLQALHSVNSFFDRNIGNLENSLKHEGHAPIPSLTTGNLTKTFLQLVEDKFPSSSKAQKALNELVGPDTGPQTEVLNLFFRALNGC
SPRIFSGAEKKQQLASVITNTLDAINADNEDYPKPGDFPRSSFSSTPPHAPVPQSEIPTSPTSTQPPSP

(3) ArtJ arginine-binding protein

One example of 'ArtJ' protein is disclosed as SEQ ID NO[s]: 105 & 106 in reference 17 {GenBank accession number: AAC67977, GI:3328806; 'CT381'; SEQ ID NO: 6 below}.

Preferred ArtJ proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 6; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 6, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These ArtJ proteins include variants (*e.g.*

allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 6. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 6. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 6. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

The ArtJ protein may be bound to a small molecule like arginine or another amino acid.

SEQ ID NO: 6

MCIKRKKTWIAFLAVVCSFCLTGCLKEGGDSNSEKFIVGTNATYPPFEFVDKRGEVVGFDIDLAREISNKLGKTLDVREFSFDALILN
LKQHRIDAVITGMSITPSRLKEILMIPYYGEEIKHLVLVFKGENKHPLPLTQYRSVAVQTGTYQEAYLQSLSEVHIRSFDSTLEVLME
VMHGKSPVAVLEPSIAQVVLKDFPALSTATIDLPEDQWVLGYGIGVASDRPALALKIEAAVQEIRKEGVLAELEQKWGLNN

(4) DnaK heat-shock protein 70 (chaperone)

One example of 'DnaK' protein is disclosed as SEQ ID NO[s]: 107 & 108 in reference 17 {GenBank accession number: AAC67993, GI:3328822; 'CT396'; SEQ ID NO: 7 below}. Other sequences are disclosed in references 18, 19 and 20.

Preferred DnaK proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 7; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 7, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These DnaK proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 7. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 7. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 7. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

The DnaK may be phosphorylated *e.g.* at a threonine or a tyrosine.

SEQ ID NO: 7

MSEKRKSNKIIGIDLGTTNSCVSVMEGGQPKVIASSEGTRTTPSIVAFKGGETLVGIPAKRQAVTNPEKTLASTKRFIGRKFSEVESE
IKTVPYKVAPNSKGDAVFDVEQKLYTPEEIGAQILMKMKETAEAYLGETVTEAVITVPAYFNDSQRASTKDAGRIAGLDVKRIIPEPT
AAALAYGIDKEGDKKIAVFDLGGGTFDISILEIGDGVFEVLSTNGDTHLGGDDFDGVIINWMLDEFKKQEGIDLSKDNMALQRLKDAA
EKAKIELSGVSSTEINQPFITIDANGPKHLALTLTRAQFEHLASSLIERTKQPCAQALKDAKLSASDIDDVLLVGGMSRMPAVQAVVK
EIFGKEPNKGVNPDEVVAIGAAIQGGVLGGEVKDVLLLDVIPLSLGIETLGGVMTPLVERNTTIPTQKKQIFSTAADNQPAVTIVVLQ
GERPMAKDNKEIGRFDLTDIPPAPRGHPQIEVTFDIDANGILHVSAKDAASGREQKIRIEASSGLKEDEIQQMIRDAELHKEEDKQRK
EASDVKNEADGMIFRAEKAVKDYHDKIPAELVKEIEEHIEKVRQAIKEDASTTAIKAASDELSTHMQKIGEAMQAQSASAAASSAANA
QGGPNINSEDLKKHSFSTRPPAGGSASSTDNIEDADVEIVDKPE

(5) CT398 protein

One example of 'CT398' protein is disclosed as SEQ ID NO[s]: 111 & 112 in reference 17 {GenBank accession number: AAC67995, GI:3328825; SEQ ID NO: 8 below}.

Preferred CT398 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 8; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 8, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These CT398 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 8. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 8. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 8. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 8

```
MHDALQSILAIQELDIKMIRLMRVKKEHQNELAKIQALKTDIRRKVEEKEQEMEKLKDQIKGGEKRIQEISDQINKLENQQAAVKKMD
EFNALTQEMTAANKERRTLEHQLSDLMDKQAGSEDLLISLKESLSSTENSSSAIEEEIRENIRKINEEGRSLLSQRTQLKETTDPELF
SIYERLLNNKKDRVVVPIENRVCSGCHIALTPQHENLVRKQDHLVFCEHCSRILYWQELQSPSAEGATTKRRRRRTAV
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(6) OmpH-like outer membrane protein

One example of 'OmpH-like' protein is disclosed as SEQ ID NO[s]: 57 & 58 in reference 17 {GenBank accession number: AAC67835, GI:3328652; 'CT242'; SEQ ID NO: 4 below}. A variant sequence is disclosed in reference 21.

Preferred OmpH-like proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 4; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 4, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmpH-like proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 4. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 4. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 19 or more, to remove the signal peptide) from the N-terminus of SEQ ID NO: 4. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 4

MKKFLLLSLMSLSSLPTFAANSTGTIGIVNLRRCLEESALGKKESAEFEKMKNQFSNSMGKMEEELSSIYSKLQDDDYMEGLSETAAA
ELRKKFEDLSAEYNTAQGQYYQILNQSNLKRMQKIMEEVKKASETVRIQEGLSVLLNEDIVLSIDSSADKTDAVIKVLDDSFQNN

(7) L7/L12 ribosomal protein

One example of 'L7/L12' protein is deposited in GenBank under accession number AAC67909 (GI:3328733; 'CT316'; SEQ ID NO: 5 below}.

Preferred L7/L12 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 5; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 5, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These L7/L12 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 5. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 5. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 5. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

The L7/L12 protein may be N-terminally modified.

SEQ ID NO: 5

MTTESLETLVEQLSGLTVLELSQLKKLLEEKWDVTAAAPVVAVAGAAAAGDAPASAEPTEFAVILEDVPSDKKIGVLKVVREVTGLAL
KEAKEMTEGLPKTVKEKTSKSDAEDTVKKLQEAGAKAVAKGL

(8) OmcA cysteine-rich lipoprotein

One example of 'OmcA' protein is disclosed as SEQ ID NO^{s}: 127 & 128 in reference 17 {GenBank accession number: AAC68043, GI:3328876; 'CT444', 'Omp2A', 'Omp3'; SEQ ID NO: 9 below}. A variant sequence is disclosed in reference 22.

Preferred OmcA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 9; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 9, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmcA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 9. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 9. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one

or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 18 or more to remove the signal peptide) from the N-terminus of SEQ ID NO: 9. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

The protein may be lipidated (*e.g.* by a *N*-acyl diglyceride), and may thus have a N-terminal cysteine.

SEQ ID NO: 9

MKKTALLAALCSVVSLSSCCRIVDCCFEDPCAPIQCSPCESKKKDVDGGCNSCNGYVPACKPCGGDTHQDAKHGPQARGIPVDGKCRQ

(9) AtoS two-component regulatory system sensor histidine kinase protein

One example of 'AtoS' protein is disclosed as SEQ ID NO[s]: 129 & 130 in reference 17 {GenBank accession number: AAC68067, GI:3328901; 'CT467'; SEQ ID NO: 10 below}.

Preferred AtoS proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 10; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 10, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These AtoS proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 10. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 10. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 10. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 10

MPKIDTCDSCVSNTELLAIRTRVTQSYNEAQTILSSIPDGIFLLSESGEILICNPQARAILGIPEDIQLVTRMFHDFFPDTFFGFSVQ
EALEKEVPPKTIRLTLSQELSQKEVEVFVRKNISHDFLFLLIRDRSDYRQLEQAIEKYRSISELGKIAATLAHEIRNPLTSISGFATL
LKEELSSERHQRMLNVIIEGTRSLNSLVSSMLEYTKIQPLNLRSIDLQDFFSSLIPELSLTFPSCTFRRTILSPIQRSIDPDRLRCVI
WNLVKNAVEASDEEIFLELHEKGFSVINTGTLPPNIQEKLFIPFFTTKPQGNGLGLAEAHKIMRLHGGDLVVSTQDNRTTFTILWTPA

(10) CT547 protein

One example of 'CT547' protein is disclosed as SEQ ID NO[s]: 151 & 152 in reference 17 {GenBank accession number: AAC67995, GI:3328825; SEQ ID NO: 11 below}.

Preferred CT547 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 11; and/or (b) which is a fragment of at least

n consecutive amino acids of SEQ ID NO: 11, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These CT547 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 11. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 11. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 11. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 11

MKVILRALCLFLVLPCGCYARVPSFEPFRGAIAPNRYTPKHSPELYFEMGDKYFQAKKFKQALLCFGMITHHFPEHALHPKAQFLVGL
CYLEMGHPDLADKALTQYQELADTEYSEQLFAIKYSIAQSFANGKRKNIVPLEGFPKLLKADTDALRIFEEIVTASSDADLKASALYA
KGALLFDRKEYSEAIKTLKKVSLQFPSHSLSPESFTLIAKIHCLQALQEPYNEQYLQDARMNAAALRKQHPNHPSNTEVENYIHHMCE
AYASCLYSTGRFYEKKRKASSAKIYYSIALENFPDTSYVAKCNKRLERLSKQMS

(11) Enolase (2-phosphoglycerate dehydratase) protein

One example of an 'Eno' protein is disclosed as SEQ ID NO[s]: 189 & 190 in reference 17 {GenBank accession number: AAC68189, GI:3329030; 'CT587'; SEQ ID NO: 12 below}.

Preferred Eno proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 12; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 12, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Eno proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 12. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 12. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 12. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

The Eno protein may contain magnesium ions, and may be in the form of a homodimer.

SEQ ID NO: 12

MFDVVISDIEAREILDSRGYPTLCVKVITNTGTFGEACVPSGASTGIKEALELRDKDPKRYQGKGVLQAISNVEKVLMPALQGFSVFD
QITADAIMIDADGTPNKEKLGANAILGVSLALAKAAANTLQRPLYRYLGGSFSHVLPCPMMNLINGGMHATNGLQFQEFMIRPISAPS
LTEAVRMGAEVFNALKKILQNRQLATGVGDEGGFAPNLASNAEALDLLLTAIETAGFTPREDISLALDCAASSFYNTQDKTYDGKSYA
DQVGILAELCEHYPIDSIEDGLAEEDFEGWKLLSETLGDRVQLVGDDLFVTNSALIAEGIAQGLANAVLIKPNQIGTLTETAEAIRLA
TIQGYATILSHRSGETEDTTIADLAVAFNTGQIKTGSLSRSERIAKYNRLMAIEEEMGPEALFQDSNPFSKA

(12) HrtA DO protease protein

One example of an 'HrtA' protein is disclosed as SEQ ID NO[s]: 229 & 230 in reference 17 {GenBank accession number: AAC68420, GI:3329293; 'CT823'; SEQ ID NO: 13 below}.

Preferred HrtA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 13; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 13, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These HrtA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 13. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 13. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably at least 16 to remove the signal peptide) from the N-terminus of SEQ ID NO: 13. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). In relation to SEQ ID NO: 13, distinct domains are residues: 1-16; 17-497; 128-289; 290-381; 394-485; and 394-497.

SEQ ID NO: 13

```
MMKRLLCVLLSTSVFSSPMLGYSASKKDSKADICLAVSSGDQEVSQEDLLKEVSRGFSRVAAKATPGVVYIENFPKTGNQAIASPGNK
RGFQENPFDYFNDEFFNRFFGLPSHREQQRPQQRDAVRGTGFIVSEDGYVVTNHHVVEDAGKIHVTLHDGQKYTAKIVGLDPKTDLAV
IKIQAEKLPFLTFGNSDQLQIGDWAIAIGNPFGLQATVTVGVISAKGRNQLHIVDFEDFIQTDAAINPGNSGGPLLNINGQVIGVNTA
IVSGSGGYIGIGFAIPSLMAKRVIDQLISDGQVTRGFLGVTLQPIDSELATCYKLEKVYGALVTDVVKGSPAEKAGLRQEDVIVAYNG
KEVESLSALRNAISLMMPGTRVVLKIVREGKTIEIPVTVTQIPTEDGVSALQKMGVRVQNITPEICKKLGLAADTRGILVVAVEAGSP
AASAGVAPGQLILAVNRQRVASVEELNQVLKNSKGENVLLMVSQGDVVRFIVLKSDE
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(13) MurG peptidoglycan transferase protein

One example of a 'MurG' protein is disclosed as SEQ ID NO[s]: 217 & 218 in reference 17 {GenBank accession number: AAC68356, GI:3329223; 'CT761'; SEQ ID NO: 14 below} It is a UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide)pyrophosphoryl-undecaprenol N-acetylglucosamine transferase.

Preferred MurG proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 14; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 14, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These MurG proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 14. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 14. Other preferred fragments lack one or

more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 14. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The MurG may be lipidated *e.g.* with undecaprenyl.

SEQ ID NO: 14

MKKINKIVLAVGGTGGHIIPALAARETFIHEDIEVLLLGKGLAHFLGDDSEVAYCDIPSGSPFSLRVNRMFSGAKQLYKGYVAALQKI
RDFTPDLAIGFGSYHSLPAMLASIRSRIPLFLHEQNIVPGKVNKLFSRFAKGVGMSFAAAGEHFHCRAEEVFLPIRKLSEQIVFPGAS
PVICVVGGSQGAKILNDVVPKALARIRESYSNLYVHHIVGPKGDLQAVSQVYQDAGINHTVTAFDHNMLGVLQASDLVISRSGATMLN
ELLWVQVPAILIPYPGAYGHQEVNAKFFTHTVGGGTMILQKYLTEESLSKQVLLALDPATSENRRKAMLSAQQKKSFKSLYQFICESL

The immunogenicity of other known *Chlamydia trachomatis* antigens may be improved by combination with two or more *Chlamydia trachomatis* antigens from either the first antigen group or the second antigen group. Such other known *Chlamydia trachomatis* antigens include a third antigen group consisting of (1) PGP3, (2) one or more PMP, (3) MOMP (CT681), (4) Cap1 (CT529); (5) GroEL-like hsp60 protein (Omp2); and (6) 60 kDa Cysteine rich protein (omcB). These antigens are referred to herein as the "third antigen group".

The invention thus includes a composition comprising a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, or five *Chlamydia trachomatis* antigens of the first antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens from the first antigen group and three, four, or five *Chlamydia trachomatis* antigens from the third antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens from the first antigen group and three, four or five *Chlamydia trachomatis* antigens from the third antigen group.

The invention further includes a composition comprising a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or thirteen *Chlamydia trachomatis* antigens of the second antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens from the second antigen group and three, four or five *Chlamydia trachomatis* from the third antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens from the second antigen group and three, four or five *Chlamydia trachomatis* antigens of the third antigen group.

In either of the above combinations, preferably the *Chlamydia trachomatis* antigens from the third antigen group include Cap 1. Or, alternatively, in either of the above combinations, preferably the *Chlamydia trachomatis* antigens from the third antigen group include MOMP.

Each of the *Chlamydia trachomatis* antigens of the third antigen group are described in more detail below.

(1) Plasmid Encoded Protein (PGP3)

One example of PGP3 sequence is disclosed in, for example, at Genbank entry GI 121541. Immunization with pgp3 is discussed in Ref. 23 and 24. One example of a PGP3 protein is set forth below as SEQ ID NO: 15.

Preferred PGP3 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 15; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 15, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PGP3 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 15. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 15. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 15. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 15

```
MGNSGFYLYNTQNCVFADNIKVGQMTEPLKDQQIILGTTSTPVAAKMTASDGISLTVSNNPSTNASITIGLDAEKAY
QLILEKLGDQILGGIADTIVDSTVQDILDKITTDPSLGLLKAFNNFPITNKIQCNGLFTPRNIETLLGGTEIGKFTV
TPKSSGSMFLVSADIIASRMEGGVVLALVREGDSKPYAISYGYSSGVPNLCSLRTRIINTGLTPTTYSLRVGGLESG
VVWVNALSNGNDILGITNTSNVSFLEVIPQTNA
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(2) Polymorphic Membrane Proteins (PMP)

A family of nine *Chlamydia trachomatis* genes encoding predicted polymorphic membrane proteins (PMP) have been identified (*pmpA* to *pmpI*). See Ref. 25, specifically Figure 1. Examples of Amino acid sequences of the PMP genes are set forth as SEQ ID NOS: 16 – 24. (These sequences can also be found at Genbank Ref. Nos. GI 15605137 (*pmpA*), 15605138 (*pmpB*), 15605139 (*pmpC*), 15605546 (*pmpD*), 15605605 (*pmpE*), 15605606 (*pmpF*), 15605607 (*pmpG*), 15605608 (*pmpH*), and 15605610 (*pmpH*)). These PMP genes encode relatively large proteins (90 to 187 kDa in mass). The majority of these PMP proteins are predicted to be outer membrane proteins, and are thus also referred to as Predicted Outer Membrane Proteins. As used herein, PMP refers to one or more of the

Chlamydia trachomatis pmp proteins (*pmpA* to *pmpI*) or an immunogenic fragment thereof. Preferably, the PMP protein used in the invention is *pmpE* or *pmpI*. Preferably, the PMP protein used in the invention comprises one or more of the fragments of *pmpE* or *pmpI* identified in International Patent Application PCT/US01/30345 (WO 02/28998) in Table 1 on page 20 (preferred fragments of *pmpE*) or Table 2 on page 21 (preferred fragments of *pmpI*).

Preferred PMP proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to one of the polypeptide sequences set forth as SEQ ID NOS: 16 - 24; and/or (b) which is a fragment of at least *n* consecutive amino acids of one of the polypeptide sequences set forth as SEQ ID NOS: 16 - 24, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PMP proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of the polypeptide sequences set forth as SEQ ID NOS: 16 - 24. Preferred fragments of (b) comprise an epitope from one of the polypeptide sequences set forth as SEQ ID NOS: 16 - 24. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of one of the polypeptide sequences set forth as SEQ ID NOS: 16 - 24. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 16 (pmpA)

```
MNRVIEIHAHYDQRQLSQSPNTNFLVHHPYLTLIPKFLLGALIVYAPYSFAEMELAISGHKQGKDRDTFT
MISSCPEGTNYIINRKLILSDFSLLNKVSSGGAFRNLAGKISFLGKNSSASIHFKHININGFGAGVFSES
SIEFTDLRKLVAFGSESTGGIFTAKEDISFKNNHHIAFRNNITKGNGGVIQLQGDMKGSVSFVDQRGAII
FTNNQAVTSSSMKHSGRGGAISGDFAGSRILFLNNQQITFEGNSAVHGGAIYNKNGLVEFLGNAGPLAFK
ENTTIANGGAIYTSNFKANQQTSPILFSQNHANKKGGAIYAQYVNLEQNQDTIRFEKNTAKEGGGAITSS
QCSITAHNTIIFSDNAAGDLGGGAILLEGKKPSLTLIAHSGNIAFSGNTMLHITKKASLDRHNSILIKEA
PYKIQLAANKNHSIHFFDPVMALSASSSPIQINAPEYETPFFSPKGMIVFSGANLLDDAREDVANRTSIF
NQPVHLYNGTLSIENGAHLIVQSFKQTGGRISLSPGSSLALYTMNSFFHGNISSKEPLEINGLSFGVDIS
PSNLQAEIRAGNAPLRLSGSPSIHDPEGLFYENRDTAASPYQMEILLTSDKIVDISKFTTDSLVTNKQSG
FQGAWHFSWQPNTINNTKQKILRASWLPTGEYVLESNRVGRAVPNSLWSTFLLLQTASHNLGDHLCNNRS
LIPTSYFGVLIGGTGAEMSTHSSEEESFISRLGATGTSIIRLTPSLTLSGGGSHMFGDSFVADLPEHITS
EGIVQNVGLTHVWGPLTVNSTLCAALDHNAMVRICSKKDHTYGKWDTFGMRGTLGASYTFLEYDQTMRVF
SFANIEATNILQRAFTETGYNPRSFSKTKLLNIAIPIGIGYEFCLGNSSFALLGKGSIGYSRDIKRENPS
TLAHLAMNDFAWTTNGCSVPTSAHTLANQLILRYKACSLYITAYTINREGKNLSNSLSCGGYVGF
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SEQ ID NO: 17 (pmpB)

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MKWLSATAVFAAVLPSVSGFCFPEPKELNFSRVGTSSSTTFTETVGEAGAEYIVSGNASFTKFTNIPTTD
TTTPTNSNSSSSNGETASVSEDSDSTTTTPDPKGGGAFYNAHSGVLSFMTRSGTEGSLTLSEIKITGEGG
AIFSQGELLFTDLTGLTIQNNLSQLSGGAIFGESTISLSGITKATFSSNSAEVPAPVKKPTEPKAQTASE
TSGSSSSSGNDSVSSPSSSRAEPAAANLQSHFICATATPAAQTDTETSTPSHKPGSGGAIYAKGDLTIAD
SQEVLFSINKATKDGGAIFAEKDVSFENITSLKVQTNGAEEKGGAIYAKGDLSIQSSKQSLFNSNYSKQG
GGALYVEGDINFQDLEEIRIKYNKAGTFETKKITLPKAQASAGNADAWASSSPQSGSGATTVSNSGDSSS
GSDSDTSETVPATAKGGGLYTDKNLSITNITGIIEIANNKATDVGGGAYVKGTLTCENSHRLQFLKNSSD
KQGGGIYGEDNITLSNLTGKTLFQENTAKEEGGGLFIKGTDKALTMTGLDSFCLINNTSEKHGGGAFVTK
EISQTYTSDVETIPGITPVHGETVITGNKSTGGNGGGVCTKRLALSNLQSISISGNSAAENGGGAHTCPD
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SFPTADTAEQPAAASAATSTPESAPVVSTALSTPSSSTVSSLTLLAASSQASPATSNKETQDPNADTDLL
IDYVVDTTISKNTAKKGGGIYAKKAKMSRIDQLNISENSATEIGGGICCKESLELDALVSLSVTENLVGK
EGGGLHAKTVNISNLKSGFSFSNNKANSSSTGVATTASAPAAAAASLQAAAAAVPSSPATPTYSGVVGGA
IYGEKVTFSQCSGTCQFSGNQAIDNNPSQSSLNVQGGAIYAKTSLSIGSSDAGTSYIFSGNSVSTGKSQT
TGQIAGGAIYSPTVTLNCPATFSNNTASMATPKTSSEDGSSGNSIKDTIGGAIAGTAITLSGVSRFSGNT
ADLGAAIGTLANANTPSATSGSQNSITEKITLENGSFIFERNQANKRGAIYSPSVSIKGNNITFNQNTST
HDGSAIYFTKDATIESLGSVLFTGNNVTATQASSATSGQNTNTANYGAAIFGDPGTTQSSQTDAILTLLA
SSGNITFSNNSLQNNQGDTPASKFCSIAGYVKLSLQAAKGKTISFFDCVHTSTKKIGSTQNVYETLDINK
EENSNPYTGTIVFSSELHENKSYIPQNAILHNGTLVLKEKTELHVVSFEQKEGSKLIMKPGAVLSNQNIA
NGALVINGLTIDLSSMGTPQAGEIFSPPELRIVATTSSASGGSGVSSSIPTNPKRISAAAPSGSAATTPT
MSENKVFLTGDLTLIDPNGNFYQNPMLGSDLDVPLIKLPTNTSDVQVYDLTLSGDLFPQKGYMGTWTLDS
NPQTGKLQARWTFDTYRRWVYIPRDNHFYANSILGSQNSMIVVKQGLINNMLNNARFDDIAYNNFWVSGV
GTFLAQQGTPLSEEFSYYSRGTSVAIDAKPRQDFILGAAFSKMVGKTKAIKKMHNYFHKGSEYSYQASVY
GGKFLYFLLNKQHGWALPFLIQGVVSYGHIKHDTTTLYPSIHERNKGDWEDLGWLADLRISMDLKEPSKD
SSKRITVYGELEYSSIRQKQFTEIDYDPRHFDDCAYRNLSLPVGCAVEGAIMNCNILMYNKLALAYMPSI
YRNNPVCKYRVLSSNEAGQVICGVPTRTSARAEYSTQLYLGPFWTLYGNYTIDVGMYTLSQMTSCGARMI
F

SEQ ID NO: 18 (pmpC)

MKFMSATAVFAAALSSVTEASSIQDQIKNTDCNVSKLGYSTSQAFTDMMLADNTEYRAADSVSFYDFSTS
SRLPRKHLSSSSEASPTTEGVSSSSSGETDEKTEEELDNGGIIYAREKLTISESQDSLSNQSIELHDNSI
FFGEGEVIFDHRVALKNGGAIYGEKEVVFENIKSLLVEVNIAVEKGGSVYAKERVSLENVTEATFSSNGG
EQGGGGIYSEQDMLISDCNNVHFQGNAAGATAVKQCLDEEMIVLLAECVDSLSEDTLDSTPETEQTESNG
NQDGSSETEDTQVSESPESTPSPDDVLGKGGGIYTEKSLTITGITGTIDFVSNIATDSGAGVFTKENLSC
TNTNSLQFLKNSAGQHGGGAYVTQTMSVTNTTSESITTPPLIGEVIFSENTAKGHGGGICTNKLSLSNLK
TVTLTKNSAKESGGAIFTDLASIPITDTPESSTPSSSSPASTPEVVASAKINRFFASTAKPAAPSLTEAE
SDQTDQTETSDTNSDIDVSIENILNVAINQNTSAKKGGAIYGKKAKLSRINNLELSGNSSQDVGGGLCLT
ESVEFDAIGSLLSHYNSAAKEGGAIHSKTVTLSNLKSTFTFADNTVKAIVESTPEAPEEIPPVEGEESTA
TEDPNSNTEGSSANTNLEGSQGDTADTGTGDVNNESQDTSDTGNAESEEQLQDSTQSNEENTLPNSNIDQ
SNENTDESSDSHTEEITDESVSSSSESGSSTPQDGGAASSGAPSGDQSISANACLAKSYAASTDSSPVSN
SSGSEEPVTSSSDSDVTASSDNPDSSSSGDSAGDSEEPTEPEAGSTTETLTLIGGGAIYGETVKIENFSG
QGIFSGNKAIDNTTEGSSSKSDVLGGAVYAKTLFNLDSGSSRRTVTFSGNTVSSQSTTGQVAGGAIYSPT
VTIATPVVFSKNSATNNANNTTDTQRKDTFGGAIGATSAVSLSGGAHFLENVADLGSAIGLVPGTQNTET
VKLESGSYYFEKNKALKRATIYAPVVSIKAYTATFNQNRSLEEGSAIYFTKEASIESLGSVLFTGNLVTL
TLSTTTEGTPATTSGDVTKYGAAIFGQIASSNGSQTDNLPLKLIASGGNICFRNNEYRPTSSDTGTSTFC
SIAGDVKLTMQAAKGKTISFFDAIRTSTKKTGTQATAYDTLDINKSEDSETVNSAFTGTILFSSELHENK
SYIPQNVVLHSGSLVLKPNTELHVISFEQKEGSSLVMTPGSVLSNQTVADGALVINNMTIDLSSVEKNGI
AEGNIFTPPELRIIDTTTGGSGGTPSTDSESNQNSDDTEEQNNNDASNQGESANGSSSPAVAAAHTSRTR
NFAAAATATPTTTPTATTTTSNQVILGGEIKLIDPNGTFFQNPALRSDQQISLLVLPTDSSKMQAQKIVL
TGDIAPQKGYTGTLTLDPDQLQNGTISVLWKFDSYRQWAYVPRDNHFYANSILGSQMLMVTVKQGLLNDK
MNLARFEEVSYNNLWISGLGTMLSQVGTPTSEEFTYYSRGASVALDAKPAHDVIVGAAFSKMIGKTKSLK
RENNYTHKGSEYSYQASVYGGKPFHFVINKKTEKSLPLLLQGVISYGYIKHDTVTHYPTIRERNKGEWED
LGWLTALRVSSVLRTPAQGDTKRITVYGELEYSSIRQKQFTETEYDPRYFDNCTYRNLAIPMGLAFEGEL
SGNDILMYNRFSVAYMLSIYRNSPTCKYQVLSSGEGGEIICGVPTRNSARGEYSTQLYLGPLWTLYGSYT
IEADAHTLAHMMNCGARMTF

SEQ ID NO: 19 (pmpD)

MSSEKDIKSTCSKFSLSVVAAILASVSGLASCVDLHAGGQSVNELVYVGPQAVLLLDQIRDLFVGSKDSQ
AEGQYRLIVGDPSSFQEKDADTLPGKVEQSTLFSVTNPVVFQGVDQQDQVSSQGLICSFTSSNLDSPRDG
ESFLGIAFVGDSSKAGITLTDVKASLSGAALYSTEDLIFEKIKGGLEFASCSSLEQGGACAAQSILIHDC
QGLQVKHCTTAVNAEGSSANDHLGFGGGAFFVTGSLSGEKSLYMPAGDMVVANCDGAISFEGNSANFANG
GAIAASGKVLFVANDKKTSFIENRALSGGAIAASSDIAFQNCAELVFKGNCAIGTEDKGSLGGGAISSLG
TVLLQGNHGITCDKNESASQGGAIFGKNCQISDNEGPVVFRDSTACLGGGAIAAQEIVSIQNNQAGISFE
GGKASFGGGIACGSFSSAGGASVLGTIDISKNLGAISFSRTLCTTSDLGQMEYQGGGALFGENISLSENA
GVLTFKDNIVKTFASNGKILGGGAILATGKVEITNNSEGISFTGNARAPQALPTQEEFPLFSKKEGRPLS
SGYSGGGAILGREVAILHNAAVVFEQNRLQCSEEEATLLGCCGGGAVHGMDSTSIVGNSSVRFGNNYAMG
QGVSGGALLSKTVQLAGNGSVDFSRNIASLGGGALQASEGNCELVDNGYVLFRDNRGRVYGGAISCLRGD
VVISGNKGRVEFKDNIATRLYVEETVEKVEEVEPAPEQKDNNELSFLGRAEQSFITAANQALFASEDGDL

SPESSISSEELAKRRECAGGAIFAKRVRIVDNQEAVVFSNNFSDIYGGAIFTGSLREEDKLDGQIPEVLI
SGNAGDVVFSGNSSKRDEHLPHTGGGAICTQNLTISQNTGNVLFYNNVACSGGAVRIEDHGNVLLEAFGG
DIVFKGNSSFRAQGSDAIYFAGKESHITALNATEGHAIVFHDALVFENLEERKSAEVLLINSRENPGYTG
SIRFLEAESKVPQCIHVQQGSLELLNGATLCSYGFKQDAGAKLVLAAGAKLKILDSGTPVQQGHAISKPE
AEIESSSEPEGAHSLWIAKNAQTTVPMVDIHTISVDLASFSSSQQEGTVEAPQVIVPGGSYVRSGELNLE
LVNTTGTGYENHALLKNEAKVPLMSFVASGDEASAEISNLSVSDLQIHVVTPEIEEDTYGHMGDWSEAKI
QDGTLVISWNPTGYRLDPQKAGALVFNALWEEGAVLSALKNARFAHNLTAQRMEFDYSTNVWGFAFGGFR
TLSAENLVAIDGYKGAYGGASAGVDIQLMEDFVLGVSGAAFLGKMDSQKFDAEVSRKGVVGSVYTGFLAG
SWFFKGQYSLGETQNDMKTRYGVLGESSASWTSRGVLADALVEYRSLVGPVRPTFYALHFNPYVEVSYAS
MKFPGFTEQGREARSFEDASLTNITIPLGMKFELAFIKGQFSEVNSLGISYAWEAYRKVEGGAVQLLEAG
FDWEGAPMDLPRQELRVALENNTEWSSYFSTVLGLTAFCGGFTSTDSKLGYEANTGLRLIF

SEQ ID NO: 20 (pmpE)

MKKAFFFFLIGNSLSGLAREVPSRIFLMPNSVPDPTKESLSNKISLTGDTHNLTNCYLDNLRYILAILQK
TPNEGAAVTITDYLSFFDTQKEGIYFAKNLTPESGGAIGYASPNSPTVEIRDTIGPVIFENNTCCRLFTW
RNPYAADKIREGGAIHAQNLYINHNHDVVGFMKNFSYVQGGAISTANTFVVSENQSCFLFMDNICIQTNT
AGKGGAIYAGTSNSFESNNCDLFFINNACCAGGAIFSPICSLTGNRGNIVFYNNRCFKNVETASSEASDG
GAIKVTTRLDVTGNRGRIFFSDNITKNYGGAIYAPVVTLVDNGPTYFINNIANNKGGAIYIDGTSNSKIS
ADRHAIIFNENIVTNVTNANGTSTSANPPRRNAITVASSSGEILLGAGSSQNLIFYDPIEVSNAGVSVSF
NKEADQTGSVVFSGATVNSADFHQRNLQTKTPAPLTLSNGFLCIEDHAQLTVNRFTQTGGVVSLGNGAVL
SCYKNGTGDSASNASITLKHIGLNLSSILKSGAEIPLLWVEPTNNSNNYTADTAATFSLSDVKLSLIDDY
GNSPYESTDLTHALSSQPMLSISEASDNQLQSENIDFSGLNVPHYGWQGLWTWGWAKTQDPEPASSATIT
DPQKANRFHRTLLLTWLPAGYVPSPKHRSPLIANTLWGNMLLATESLKNSAELTPSGHPFWGITGGGLGM
MVYQDPRENHPGFHMRSSGYSAGMIAGQTHTFSLKFSQTYTKLNERYAKNNVSSKNYSCQGEMLFSLQEG
FLLTKLVGLYSYGDHNCHHFYTQGENLTSQGTFRSQTMGGAVFFDLPMKPFGSTHILTAPFLGALGIYSS
LSHFTEVGAYPRSFSTKTPLINVLVPIGVKGSFMNATHRPQAWTVELAYQPVLYRQEPGIAAQLLASKGI
WFGSGSPSSRHAMSYKISQQTQPLSWLTLHFQYHGFYSSSTFCNYLNGEIALRF

SEQ ID NO: 21 (pmpF)

MIKRTSLSFACLSFFYLSTISILQANETDTLQFRRFTFSDREIQFVLDPASLITAQNIVLSNLQSNGTGA
CTISGNTQTQIFSNSVNTTADSGGAFDMVTTSFTASDNANLLFCNNYCTHNKGGGAIRSGGPIRFLNNQD
VLFYNNISAGAKYVGTGDHNEKNRGGALYATTITLTGNRTLAFINNMSGDCGGAISADTQISITDTVKGI
LFENNHTLNHIPYTQAENMARGGAICSRRDLCSISNNSGPIVFNYNQGGKGGAISATRCVIDNNKERIIF
SNNSSLGWSQSSSASNGGAIQTTQGFTLRNNKGSIYFDSNTATHAGGAINCGYIDIRDNGPVYFLNNSAA
WGAAFNLSKPRSATNYIHTGTGDIVFNNNVVFTLDGNLLGKRKLFHINNNEITPYTLSLGAKKDTRIYFY
DLFQWERVKENTSNNPPSPTSRNTITVNPETEFSGAVVFSYNQMSSDIRTLMGKEHNYIKEAPTTLKFGT
LAIEDDAELEIFNIPFTQNPTSLLALGSGATLTVGKHGKLNITNLGVILPIILKEGKSPPCIRVNPQDMT
QNTGTGQTPSSTSSISTPMIIFNGRLSIVDENYESVYDSMDLSRGKAEQLILSIETTNDGQLDSNWQSSL
NTSLLSPPHYGYQGLWTPNWITTTYTYITLNNNSSAPTSATSIAEQKKTSETFTPSNTTTASIPNIKASAG
SGSGSASNSGEVTITKHTLVVNWAPVGYIVDPIRRGDLIANSLVHSGRNMTMGLRSLLPDNSWFALQGAA
TTLFTKQQKRLSYHGYSSASKGYTVSSQASGAHGHKFLLSFSQSSDKMKEKETNNRLSSRYYLSALCFEH
PMFDRIALIGAAACNYGTHNMRSFYGTKKSSKGKFHSTTLGASLRCELRDSMPLRSIMLTPFAQALFSRT
EPASIRESGDLARLFTLEQAHTAVVSPIGIKGAYSSDTWPTLSWEMELAYQPTLYWKRPLLNTLLIQNNG
SWVTTNTPLAKHSFYGRGSHSLKFSHLKLFANYQAEVATSTVSHYINAGGALVF

SEQ ID NO: 22 (pmpG)

MQTSFHKFFLSMILAYSCCSLSGGGYAAEIMIPQGIYDGETLTVSFPYTVIGDPSGTTVFSAGELTLKNL
DNSIAALPLSCFGNLLGSFTVLGRGHSLTFENIRTSTNGAALSDSANSGLFTIEGFKELSFSNCNSLLAV
LPAATTNNGSQTPTTTSTPSNGTIYSKTDLLLLNNEKFSFYSNLVSGDGGAIDAKSLTVQGISKLCVFQE
NTAQADGGACQVVTSFSAMANEAPIAFIANVAGVRGGGIAAVQDGQQGVSSSTSTEDPVVSFSRNTAVEF
DGNVARVGGGIYSYGNVAFLNNGKTLFLNNVASPVYIAAEQPTNGQASNTSDNYGDGGAIFCKNGAQAAG
SNNSGSVSFDGEGVVFFSSNVAAGKGGAIYAKKLSVANCGPVQFLGNIANDGGAIYLGESGELSLSADYG
DIIFDGNLKRTAKENAADVNGVTVSSQAISMGSGGKITTLRAKAGHQILFNDPIEMANGNNQPAQSSEPL
KINDGEGYTGDIVFANGNSTLYQNVTIEQGRIVLREKAKLSVNSLSQTGGSLYMEAGSTLDFVTPQPPQQ
PPAANQLITLSNLHLSLSSLLANNAVTNPPTNPPAQDSHPAIIGSTTAGSVTISGPIFFEDLDDTAYDRY
DWLGSNQKIDVLKLQLGTQPSANAPSDLTLGNEMPKYGYQGSWKLAWDPNTANNGPYTLKATWTKTGYNP
GPERVASLVPNSLWGSILDIRSAHSAIQASVDGRSYCRGLWVSGVSNFFYHDRDALGQGYRYISGGYSLG

ANSYFGSSMFGLAFTEVFGRSKDYVVCRSNHHACIGSVYLSTKQALCGSYLFGDAFIRASYGFGNQHMKT
SYTFAEESDVRWDNNCLVGEIGVGLPIVITPSKLYLNELRPFVQAEFSYADHESFTEEGDQARAFRSGHL
MNLSVPVGVKFDRCSSTHPNKYSFMGAYICDAYRTISGTQTTLLSHQETWTTDAFHLARHGVIVRGSMYA
SLTSNIEVYGHGRYEYRDTSRGYGLSAGSKVRF

SEQ ID NO: 23 (pmpH)

MPFSLRSTSFCFLACLCSYSYGFASSPQVLTPNVTTPFKGDDVYLNGDCAFVNVYAGAENGSIISANGDN
LTITGQNHTLSFTDSQGPVLQNYAFISAGETLTLKDFSSLMFSKNVSCGEKGMISGKTVSISGAGEVIFW
DNSVGYSPLSIVPASTPTPPAPAPAPAASSSLSPTVSDARKGSIFSVETSLEISGVKKGVMFDNNAGNFG
TVFRGNSNNNAGSGGSGSATTPSFTVKNCKGKVSFTDNVASCGGGVVYKGTVLFKDNEGGIFFRGNTAYD
DLGILAATSRDQNTETGGGGGVICSPDDSVKFEGNKGSIVFDYNFAKGRGGSILTKEFSLVADDSVVFSN
NTAEKGGGAIYAPTIDISTNGGSILFERNRAAEGGAICVSEASSGSTGNLTLSASDGDIVFSGNMTSDRP
GERSAARILSDGTTVSLNASGLSKLIFYDPVVQNNSAAGASTPSPSSSSMPGAVTINQSGNGSVIFTAES
LTPSEKLQVLNSTSNFPGALTVSGGELVVTEGATLTTGTITATSGRVTLGSGASLSAVAGAANNNYTCTV
SKLGIDLESFLTPNYKTAILGADGTVTVNSGSTLDLVMESEAEVYDNPLFVGSLTIPFVTLSSSSASNGV
TKNSVTINDADAAHYGYQGSWSADWTKPPLAPDAKGMVPPNTNNTLYLTWRPASNYGEYRLDPQRKGELV
PNSLWVAGSALRTFTNGLKEHYVSRDVGFVASLHALGDYILNYTQDDRDGFLARYGGFQATAASHYENGS
IFGVAFGQLYGQTKSRMYYSKDAGNMTMLSCFGRSYVDIKGTETVMYWETAYGYSVHRMHTQYFNDKTQK
FDHSKCHWHNNNYYAFVGAEHNFLEYCIPTRQFARDYELTGFMRFEMAGGWSSSTRETGSLTRYFARGSG
HNMSLPIGIVAHAVSHVRRSPPSKLTLNMGYRPDIWRVTPHCNMEIIANGVKTPIQGSPLARHAFFLEVH
DTLYIHHFGRAYMNYSLDARRRQTAHFVSMGLNRIF

SEQ ID NO: 24 (pmpI)

MRPDHMNFCCLCAAILSSTAVLFGQDPLGETALLTKNPNHVVCTFFEDCTMESLFPALCAHASQDDPLYV
LGNSYCWFVSKLHITDPKEALFKEKGDLSIQNFRFLSFTDCSSKESSPSIIHQKNGQLSLRNNGSMSFCR
NHAEGSGGAISADAFSLQHNYLFTAFEENSSKGNGGAIQAQTFSLSRNVSPISFARNRADLNGGAICCSN
LICSGNVNPLFFTGNSATNGGAICCISDLNTSEKGSLSLACNQETLFASNSAKEKGGAIYAKHMVLRYNG
PVSFINNSAKIGGAIAIQSGGSLSILAGEGSVLFQNNSQRTSDQGLVRNAIYLEKDAILSSLEARNGDIL
FFDPIVQESSSKESPLPSSLQASVTSPTPATASPLVIQTSANRSVIFSSERLSEEEKTPDNLTSQLQQPI
ELKSGRLVLKDRAVLSAPSLSQDPQALLIMEAGTSLKTSSDLKLATLSIPLHSLDTEKSVTIHAPNLSIQ
KIFLSNSGDENFYENVELLSKEQNNIPLLTLSKEQSHLHLPDGNLSSHFGYQGDWTFSWKDSDEGHSLIA
NWTPKNYVPHPERQSTLVANTLWNTYSDMQAVQSMINTIAHGGAYLFGTWGSAVSNLFYAHDSSGKPIDN
WHHRSLGYLFGISTHSLDDHSFCLAAGQLLGKSSDSFITSTETTSYIATVQAQLATPLMKISAQACYNES
IHELKTKYRSFSKEGFGSWHSVAVSGEVCASIPIVSNGSGLFSSFSIFSKLQGFSGTQDGFEESSGEIRS
FSASSFRNISLPMGITFEKKSQKTRNYYYFLGAYIQDLKRDVESGPVVLLKNAVSWDAPMANLDSRAYMF
RLTNQRALHRLQTLLNVSYVLRGQSHSYSLDLGTTYRF

(3) Major Outer Membrane Protein (MOMP) (CT681)

One example of a MOMP sequence is disclosed as SEQ ID NOS 155 and 156 in International Patent Application No. PCT/IB02/05761 (WO 03/049762). The polypeptide sequence encoding MOMP is set forth below as SEQ ID NO: 25. This protein is thought to function *in vivo* as a porin {ref. 26}, and to be present during the whole life cycle of the bacteria {ref. 27}. MOMP displays four variable domains (VD) surrounded by five constant regions that are highly conserved among serovars {ref. 28 29}. *In vitro* and *in vivo* neutralizing B-cell epitopes have been mapped on VDs {Ref. 30, 31, 32, 33, 34}. T-cell epitopes have been identified in both variable and constant domains {35, 36}.

Preferred MOMP proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 25; and/or (b) which is a fragment of at least

n consecutive amino acids of SEQ ID NO: 25, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These MOMP proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 25. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 25, preferably one or more of the B cell or T cell epitopes identified above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 25. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). Other preferred fragments include one or more of the conserved constant regions identified above.

SEQ ID NO: 25 (MOMP)(CT681)

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MKKLLKSVLVFAALSSASSLQALPVGNPAEPSLMIDGILWEGFGGDPCDPCATWCDAISMRVGYYGDFVF
DRVLKTDVNKEFQMGAKPTTDTGNSAAPSTLTARENPAYGRHMQDAEMFTNAACMALNIWDRFDVFCTLG
ATSGYLKGNSASFNLVGLFGDNENQKTVKAESVPNMSFDQSVVELYTDTTFAWSVGARAALWECGCATLG
ASFQYAQSKPKVEELNVLCNAAEFTINKPKGYVGKEFPLDLTAGTDAATGTKDASIDYHEWQASLALSYR
LNMFTPYIGVKWSRASFDADTIRIAQPKSATAIFDTTTLNPTIAGAGDVKTGAEGQLGDTMQIVSLQLNK
MKSRKSCGIAVGTTIVDADKYAVTVETRLIDERAAHVNAQFRF
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(4) Cap1 (CT529)

The *Chlamydia trachomatis* Cap1 protein corresponds with the hypothetical open reading frame CT 529 and refers to Class I Accessible Protein-1. See Ref. 37. One example of a Cap1 protein is set forth herein as SEQ ID NO: 26. Predicted T-cell epitopes of Cap1 are identified in this reference as CSFIGGITYL, preferably SFIGGITYL, and SIIGGITYL.

Preferred Cap1 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 26; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 26, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Cap1 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 26. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 26. Preferred T-cell epitopes include one or more of the T-cell epitopes identified above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 26. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 26 (Cap1)(CT529)

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MASICGRLGSGTGNALKAFFTQPNNKMARVVNKTKGMDKTIKVAKSAAELTANILEQAGGAGSSAHITAS
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QVSKGLGDARTVVALGNAFNGALPGTVQSAQSFFSHMKAASQKTQEGDEGLTADLCVSHKRRAAAAVCSI
IGGITYLATFGAIRPILFVNKMLAKPFLSSQTKANMGSSVSYIMAANHAASVVGAGLAISAERADCEARC
ARIAREESLLEVPGEENACEKKVAGEKAKTFTRIKYALLTMLEKFLECVADVFKLVPLPITMGIRAIVAA
GCTFTSAIIGLCTFCARA

(5) GroEL-like hsp60 protein

One example of a *Chlamydia trachomatis* GroEL-like hsp60 protein is set forth herein as SEQ ID NO: 27. The role of Hsp60 in chlamydial infection is further described in, for example, 38, 39, 40, 41, and 42. Immunization of guinea pig models with recombinant Hsp60 is described in 43. B-cell epitopes of Hsp60 are identified in 44.

Preferred hsp60 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 27; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 27, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hsp60 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 27. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 27, including one or more of the epitopes identified in the references discussed above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 27. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). Other preferred fragments comprise a polypeptide sequence which does not cross-react with related human proteins.

SEQ ID NO: 27 (groEL-like hsp60 protein)

MVAKNIKYNEEARKKIQKGVKTLAEAVKVTLGPKGRHVVIDKSFGSPQVTKDGVTVAKEVELADKHENMG
AQMVKEVASKTADKAGDGTTTATVLAEAIYTEGLRNVTAGANPMDLKRGIDKAVKVVVDQIRKISKPVQH
HKEIAQVATISANNDAEIGNLIAEAMEKVGKNGSITVEEAKGFETVLDIVEGMNFNRGYLSSYFATNPET
QECVLEDALVLIYDKKISGIKDFLPVLQQVAESGRPLLIIAEDIEGEALATLVVNRIRGGFRVCAVKAPG
FGDRRKAMLEDIAILTGGQLISEELGMKLENANLAMLGKAKKVIVSKEDTTIVEGMGEKEALEARCESIK
KQIEDSSSDYDKEKLQERLAKLSGGVAVIRVGAATEIEMKEKKDRVDDAQHATIAAVEEGILPGGGTALI
RCIPTLEAFLPMLTNEDEQIGARIVLKALSAPLKQIAANAGKEGAIIFQQVMSRSANEGYDALRDAYTDM
LEAGILDPAKVTRSALESAASVAGLLLTTEALIAEIPEEKPAAAPAMPGAGMDY

(6) 60 kDa Cysteine rich protein (OmcB) (CT443)

One example of a *Chlamydia trachomatis* 60kDa Cysteine rich protein is set forth herein as SEQ ID NO: 28. This protein is also generally referred to as OmcB, Omp2 or CT 443. The role of OmcB in chlamydial infection is further described in, for example, 45, 46, 47, 48, and 49.

Preferred OmcB proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 28; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 28, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmcB proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 28. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 28, including one or more of the epitopes identified in the references discussed above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 28. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 28 (omp2/omcB)

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MRIGDPMNKLIRRAVTIFAVTSVASLFASGVLETSMAESLSTNVISLADTKAKDNTSHKSKKARKNHSKE
TPVDRKEVAPVHESKATGPKQDSCFGRMYTVKVNDDRNVEITQAVPEYATVGSPYPIEITATGKRDCVDV
IITQQLPCEAEFVRSDPATTPTADGKLVWKIDRLGQGEKSKITVWVKPLKEGCCFTAATVCACPEIRSVT
KCGQPAICVKQEGPENACLRCPVVYKINIVNQGTATARNVVVENPVPDGYAHSSGQRVLTFTLGDMQPGE
HRTITVEFCPLKRGRATNIATVSYCGGHKNTASVTTVINEPCVQVSIAGADWSYVCKPVEYVISVSNPGD
LVLRDVVVEDTLSPGVTVLEAAGAQISCNKVVWTVKELNPGESLQYKVLVRAQTPGQFTNNVVVKSCSDC
GTCTSCAEATTYWKGVAATHMCVVDTCDPVCVGENTVYRICVTNRGSAEDTNVSLMLKFSKELQPVSFSG
PTKGTITGNTVVFDSLPRLGSKETVEFSVTLKAVSAGDARGEAILSSDTLTVPVSDTENTHIY
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There is an upper limit to the number of *Chlamydia trachomatis* antigens which will be in the compositions of the invention. Preferably, the number of *Chlamydia trachomatis* antigens in a composition of the invention is less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, or less than 3. Still more preferably, the number of *Chlamydia trachomatis* antigens in a composition of the invention is less than 6, less than 5, or less than 4.

The *Chlamydia trachomatis* antigens used in the invention are preferably isolated, i.e., separate and discrete, from the whole organism with which the molecule is found in nature or, when the polynucleotide or polypeptide is not found in nature, is sufficiently free of other biological macromolecules so that the polynucleotide or polypeptide can be used for its intended purpose.

Fusion proteins

The *Chlamydia trachomatis* antigens used in the invention may be present in the composition as individual separate polypeptides, but it is preferred that at least two (*i.e.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) of the antigens are expressed as a single polypeptide chain (a

'hybrid' polypeptide). Hybrid polypeptides offer two principal advantages: first, a polypeptide that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two polypeptides which are both antigenically useful.

The hybrid polypeptide may comprise two or more polypeptide sequences from the first antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from a *Chlamydia trachomatis* antigen or a fragment thereof of the first antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise different epitopes.

The hybrid polypeptide may comprise two or more polypeptide sequences from the second antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from a *Chlamydia trachomatis* antigen or a fragment thereof of the second antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the first antigen group and one or more polypeptide sequences from the second antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the first antigen group and said second amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the second antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the first antigen group and one or more polypeptide sequences from the third antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the first antigen group and said second amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the third antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the second antigen group and one or more polypeptide sequences from the third antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the second antigen group and said second amino acid sequence selected from

a *Chlamydia trachomatis* antigen or a fragment thereof from the third antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

Hybrids consisting of amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten *Chlamydia trachomatis* antigens are preferred. In particular, hybrids consisting of amino acid sequences from two, three, four, or five *Chlamydia trachomatis* antigens are preferred.

Different hybrid polypeptides may be mixed together in a single formulation. Within such combinations, a *Chlamydia trachomatis* antigen may be present in more than one hybrid polypeptide and/or as a non-hybrid polypeptide. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both.

Two-antigen hybrids for use in the invention may comprise: (1) PepA & LcrE; (2) PepA & OmpH-like; (3) PepA & L7/L12; (4) PepA & ArtJ; (5) PepA & DnaK; (6) PepA & CT398; (7) PepA & OmcA; (8) PepA & AtoS; (9) PepA & CT547; (10) PepA & Eno; (11) PepA & HrtA; (12) PepA & MurG; (13) LcrE & OmpH-like; (14) LcrE & L7/L12; (15) LcrE & ArtJ; (16) LcrE & DnaK; (17) LcrE & CT398; (18) LcrE & OmcA; (19) LcrE & AtoS; (20) LcrE & CT547; (21) LcrE & Eno; (22) LcrE & HrtA; (23) LcrE & MurG; (24) OmpH-like & L7/L12; (25) OmpH-like & ArtJ; (26) OmpH-like & DnaK; (27) OmpH-like & CT398; (28) OmpH-like & OmcA; (29) OmpH-like & AtoS; (30) OmpH-like & CT547; (31) OmpH-like & Eno; (32) OmpH-like & HrtA; (33) OmpH-like & MurG; (34) L7/L12 & ArtJ; (35) L7/L12 & DnaK; (36) L7/L12 & CT398; (37) L7/L12 & OmcA; (38) L7/L12 & AtoS; (39) L7/L12 & CT547; (40) L7/L12 & Eno; (41) L7/L12 & HrtA; (42) L7/L12 & MurG; (43) ArtJ & DnaK; (44) ArtJ & CT398; (45) ArtJ & OmcA; (46) ArtJ & AtoS; (47) ArtJ & CT547; (48) ArtJ & Eno; (49) ArtJ & HrtA; (50) ArtJ & MurG; (51) DnaK & CT398; (52) DnaK & OmcA; (53) DnaK & AtoS; (54) DnaK & CT547; (55) DnaK & Eno; (56) DnaK & HrtA; (57) DnaK & MurG; (58) CT398 & OmcA; (59) CT398 & AtoS; (60) CT398 & CT547; (61) CT398 & Eno; (62) CT398 & HrtA; (63) CT398 & MurG; (64) OmcA & AtoS; (65) OmcA & CT547; (66) OmcA & Eno; (67) OmcA & HrtA; (68) OmcA & MurG; (69) AtoS & CT547; (70) AtoS & Eno; (71) AtoS & HrtA; (72) AtoS & MurG; (73) CT547 & Eno; (74) CT547 & HrtA; (75) CT547 & MurG; (76) Eno & HrtA; (77) Eno & MurG; or (78) HrtA & MurG.

Hybrid polypeptides can be represented by the formula **NH_2-A-{-X-L-}$_n$-B-COOH**, wherein: X is an amino acid sequence of a *Chlamydia trachomatis* antigen or a fragment thereof from the first antigen group, the second antigen group or the third antigen group; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and *n* is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the

-X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of X_2 ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

For each *n* instances of {-X-L-}, linker amino acid sequence -L- may be present or absent. For instance, when *n*=2 the hybrid may be NH_2-X_1-L_1-X_2-L_2-COOH, NH_2-X_1-X_2-COOH, NH_2-X_1-L_1-X_2-COOH, NH_2-X_1-X_2-L_2-COOH, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising Gly_n where n = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG (SEQ ID 1), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the $(Gly)_4$ tetrapeptide being a typical poly-glycine linker.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X_1 lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

Most preferably, *n* is 2 or 3.

The invention also provides nucleic acid encoding hybrid polypeptides of the invention. Furthermore, the invention provides nucleic acid which can hybridise to this nucleic acid, preferably under "high stringency" conditions (*e.g.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Polypeptides of the invention can be prepared by various means (*e.g.* recombinant expression, purification from cell culture, chemical synthesis, *etc.*) and in various forms (*e.g.* native, fusions,

non-glycosylated, lipidated, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other chlamydial or host cell proteins).

Nucleic acid according to the invention can be prepared in many ways (*e.g.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself, *etc.*) and can take various forms (*e.g.* single stranded, double stranded, vectors, probes, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other chlamydial or host cell nucleic acids).

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones (*e.g.* phosphorothioates, *etc.*), and also peptide nucleic acids (PNA), *etc.* The invention includes nucleic acid comprising sequences complementary to those described above (*e.g.* for antisense or probing purposes).

The invention also provides a process for producing a polypeptide of the invention, comprising the step of culturing a host cell transformed with nucleic acid of the invention under conditions which induce polypeptide expression.

The invention provides a process for producing a polypeptide of the invention, comprising the step of synthesising at least part of the polypeptide by chemical means.

The invention provides a process for producing nucleic acid of the invention, comprising the step of amplifying nucleic acid using a primer-based amplification method (*e.g.* PCR).

The invention provides a process for producing nucleic acid of the invention, comprising the step of synthesising at least part of the nucleic acid by chemical means.

Strains

Preferred polypeptides of the invention comprise an amino acid sequence found in *C.trachomatis* serovar D, or in one or more of an epidemiologically prevalent serotype.

Where hybrid polypeptides are used, the individual antigens within the hybrid (*i.e.* individual -X- moieties) may be from one or more strains. Where n=2, for instance, X_2 may be from the same strain as X_1 or from a different strain. Where n=3, the strains might be (i) $X_1=X_2=X_3$ (ii) $X_1=X_2\neq X_3$ (iii) $X_1\neq X_2=X_3$ (iv) $X_1\neq X_2\neq X_3$ or (v) $X_1=X_3\neq X_2$, *etc.*

Heterologous host

Whilst expression of the polypeptides of the invention may take place in *Chlamydia*, the invention preferably utilises a heterologous host. The heterologous host may be prokaryotic (*e.g.* a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis, Vibrio cholerae, Salmonella typhi, Salmonella typhimurium, Neisseria lactamica, Neisseria cinerea, Mycobacteria* (*e.g. M.tuberculosis*), yeasts, *etc.*

Immunogenic compositions and medicaments

Compositions of the invention are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of the composition is preferably between 6 and 8, preferably about 7. The pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen-free. The composition may be isotonic with respect to humans.

Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic. Accordingly, the invention includes a method for the therapeutic or prophylactic treatment of *Chlamydia trachomatis* infection in an animal susceptible to chlamydial infection comprising administering to said animal a therapeutic or prophylactic amount of the immunogenic compositions of the invention. Preferably, the immunogenic composition comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, or all five *Chlamydia trachomatis* antigens of the first antigen group. Still more preferably, the combination consists of all five *Chlamydia trachomatis* antigens of the first antigen group.

Alternatively, the immunogenic composition comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen *Chlamydia trachomatis* antigens selected from the second antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens selected from the second antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens selected from the second antigen group.

Alternatively, the immunogenic composition comprises a combination of *Chlamydia trachomatis* antigens, said combination consisting of two, three, four, or five *Chlamydia trachomatis* antigens of the first antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group. Preferably, the combination consists of three, four or five *Chlamydia trachomatis* antigens of the first antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group.

Alternatively, the immunigenic composition comprises a combination of *Chlamydia trachomatis* antigens, said combination consisting of two, three, four ,five, six, seven, eight, nine, ten, eleven, twelve or thirteen *Chlamydia trachomatis* antigens of the second antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens from the second antigen group and three, four or five *Chlamydia trachomatis* from the third antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis*

antigens from the second antigen group and three, four or five *Chlamydia trachomatis* antigens of the third antigen group.

The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of the compositions of the invention in the manufacture of a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine.

The invention also provides for a kit comprising a first component comprising a combination of *Chlamydia trachomatis* antigens. The combination of *Chlamydia trachomatis* antigens may be one or more of the immunogenic compositions of the invention. The kit may further include a second component comprising one or more of the following: instructions, syringe or other delivery device, adjuvant, or pharmaceutically acceptable formulating solution.

The invention also provides a delivery device pre-filled with the immunogenic compositions of the invention.

The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective and preferably involves antibodies and/or cell-mediated immunity. The method may raise a booster response.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant) or a teenager; where the vaccine is for therapeutic use, the human is preferably a teenager or an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

These uses and methods are preferably for the prevention and/or treatment of a disease caused by a *Chlamydia* (*e.g.* trachoma, pelvic inflammatory disease, epididymitis, infant pneumonia, *etc.*). The compositions may also be effective against *C.pneumoniae.*

One way of checking efficacy of therapeutic treatment involves monitoring *C.trachomatis* infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against the *Chlamydia trachomatis* antigens in the compositions of the invention after administration of the composition.

The vaccine compositions of the present invention can be evaluated in *in vitro* and *in vivo* animal models prior to host, e.g., human, administration. For example, *in vitro* neutralization by Peterson et al (1988) is suitable for testing vaccine compositions directed toward *Chlamydia trachomatis.*

One example of such an *in vitro* test is described as follows. Hyper-immune antisera is diluted in PBS containing 5% guinea pig serum, as a complement source. *Chlamydia trachomatis* (10^4 IFU; inclusion forming units) are added to the antisera dilutions. The antigen-antibody mixtures are incubated at 37°C for 45 minutes and inoculated into duplicate confluent Hep-2 or HeLa cell monolayers contained in glass vials (e.g., 15 by 45 mm), which have been washed twice with PBS prior to inoculation. The monolayer cells are infected by centrifugation at 1000X g for 1 hour followed by stationary incubation at 37°C for 1 hour. Infected monolayers are incubated for 48 or 72 hours , fixed and stained with Chlamydia specific antibody, such as anti-MOMP. Inclusion-bearing cells are counted in ten fields at a magnification of 200X. Neutralization titer is assigned on the dilution that gives 50% inhibition as compared to control monolayers/IFU.

The efficacy of vaccine compositions can also be determined *in vivo* by challenging animal models of *Chlamydia trachomatis* infection, *e.g.*, guinea pigs or mice, with the vaccine compositions. For example, *in vivo* vaccine composition challenge studies in the guinea pig model of *Chlamydia trachomatis* infection can be performed. A description of one example of this type of approach follows. Female guinea pigs weighing 450 – 500 g are housed in an environmentally controlled room with a 12 hour light-dark cycle and immunized with vaccine compositions via a variety of immunization routes. Post-vaccination, guinea pigs are infected in the genital tract with the agent of guinea pig inclusion conjunctivitis (GPIC), which has been grown in HeLa or McCoy cells (Rank et al. (1988)). Each animal receives approximately $1.4x10^7$ inclusion forming units (IFU) contained in 0.05 ml of sucrose-phosphate-glutamate buffer, pH 7.4 (Schacter, 1980). The course of infection monitored by determining the percentage of inclusion-bearing cells by indirect immunofluorescence with GPIC specific antisera, or by Giemsa-stained smear from a scraping from the genital tract (Rank et al 1988). Antibody titers in the serum is determined by an enzyme-linked immunosorbent assay.

Alternatively, *in vivo* vaccine compositions challenge studies can be performed in the murine model of *Chlamydia trachomatis* (Morrison et al 1995). A description of one example of this type of approach is as follows. Female mice 7 to 12 weeks of age receive 2.5 mg of depoprovera subcutaneously at 10 and 3 days before vaginal infection. Post-vaccination, mice are infected in the genital tract with 1,500 inclusion-forming units of *Chlamydia trachomatis* contained in 5ml of sucrose-phosphate-glutamate buffer, pH 7.4. The course of infection is monitored by determining the percentage of inclusion-bearing cells by indirect immunofluorescence with *Chlamydia trachomatis* specific antisera, or by a Giemsa-stained smear from a scraping from the genital tract of an infected mouse. The presence of antibody titers in the serum of a mouse is determined by an enzyme-linked immunosorbent assay.

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral (*e.g.* tablet, spray), vaginal,

topical, transdermal {*e.g.* see ref. 50} or transcutaneous {*e.g.* see refs. 51 & 52}, intranasal {*e.g.* see ref. 53}, ocular, aural, pulmonary or other mucosal administration.

The invention may be used to elicit systemic and/or mucosal immunity.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. In a multiple dose schedule the various doses may be given by the same or different routes *e.g.* a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, *etc.*

Chlamydial infections affect various areas of the body and so the compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is reconstituted just prior to administration to a patient. Such kits may comprise one or more antigens in liquid form and one or more lyophilised antigens.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Further components of the composition

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of

ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. A thorough discussion of pharmaceutically acceptable excipients is available in reference 54.

Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant.

Preferred further adjuvants include, but are not limited to, one or more of the following set forth below:

A. Mineral Containing Compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphoshpates, orthophosphates), sulphates, *etc.* {*e.g.* see chapters 8 & 9 of ref. 55}), or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt. See ref. 56.

B. Oil-Emulsions

Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). See ref. 57.

Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used as adjuvants in the invention.

C. Saponin Formulations

Saponin formulations, may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsaprilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs.

Saponin compositions have been purified using High Performance Thin Layer Chromatography (HP-LC) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in U.S. Patent No. 5,057,540. Saponin formulations may also comprise a sterol, such as cholesterol (see WO 96/33739).

Combinations of saponins and cholesterols can be used to form unique particles called Immunostimulating Complexs (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quil A, QHA and QHC. ISCOMs are further described in EP 0 109 942, WO 96/11711 and WO 96/33739. Optionally, the ISCOMS may be devoid of additional detergent. See ref. 58.

A review of the development of saponin based adjuvants can be found at ref. 59.

C. Virosomes and Virus Like Particles (VLPs)

Virosomes and Virus Like Particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO 03/024480, WO 03/024481, and Refs. 60, 61, 62 and 63. Virosomes are discussed further in, for example, Ref. 64

D. Bacterial or Microbial Derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as:

(1) *Non-toxic derivatives of enterobacterial lipopolysaccharide (LPS)*

Such derivatives include Monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP 0 689 454. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 micron membrane (see EP 0 689 454). Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529. See Ref. 65.

(2) *Lipid A Derivatives*

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in Ref. 66 and 67.

(3) *Immunostimulatory oligonucleotides*

Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a sequence containing an unmethylated cytosine followed by guanosine and linked by a phosphate bond). Bacterial double stranded RNA or oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. Optionally, the guanosine may be replaced with an analog such as 2'-deoxy-7-deazaguanosine. See ref. 68, WO 02/26757 and WO 99/62923 for examples of possible analog substitutions. The adjuvant effect of CpG oligonucleotides is further discussed in Refs. 69, 70, WO 98/40100, U.S. Patent No. 6,207,646, U.S. Patent No. 6,239,116, and U.S. Patent No. 6,429,199.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. See ref. 71. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 72, 73 and WO 01/95935. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 74, 75, 76 and WO 03/035836.

(4) *ADP-ribosylating toxins and detoxified derivatives thereof.*

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (i.e., E. coli heat labile enterotoxin "LT), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO 95/17211 and as parenteral adjuvants in WO 98/42375. The toxin or toxoid is preferably in the form of a holotoxin, comprising both A and B subunits. Preferably, the A subunit contains a detoxifying mutation; preferably the B subunit is not mutated. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LTR192G. The use of ADP-ribosylating toxins and detoxified derivaties thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in Refs. 77, 78, 79, 80, 81, 82, 83 and 84 each of which is specifically incorporated by reference herein in their entirety. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in Domenighini et al., Mol. Microbiol (1995) 15(6):1165 – 1167, specifically incorporated herein by reference in its entirety.

E. Human Immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, and tumor necrosis factor.

F. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres (Ref. 85) or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention. E.g., ref. 86.

G. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

H. Liposomes

Examples of liposome formulations suitable for use as adjuvants are described in U.S. Patent No. 6,090,406, U.S. Patent No. 5,916,588, and EP 0 626 169.

I. Polyoxyethylene ether and Polyoxyethylene Ester Formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters. Ref. 87. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol (Ref. 88) as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol (Ref. 89).

Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

J. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in Ref. 90 and 91.

K. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

L. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues, described further in Ref. 92 and 93.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

(1) a saponin and an oil-in-water emulsion (ref. 94);

(2) a saponin (e.g.., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) (see WO 94/00153);

(3) a saponin (e.g.., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) + a cholesterol;

(4) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) (Ref. 95);

combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (Ref. 96);

(5) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion.

(6) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and

(7) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML).

Aluminium salts and MF59 are preferred adjuvants for parenteral immunisation. Mutant bacterial toxins are preferred mucosal adjuvants.

The composition may include an antibiotic.

Further antigens

The compositions of the invention may further comprise antigen derived from one or more sexually transmitted diseases in addition to *Chlamydia trachomatis*. Preferably the antigen is derived from one or more of the following sexually transmitted diseases: *N.gonorrhoeae* {*e.g.* 97, 98, 99, 100}; human papiloma virus; *Treponema pallidum;* herpes simplex virus (HSV-1 or HSV-2); HIV (HIV-1 or HIV-2); and *Haemophilus ducreyi.*

A preferred composition comprises: (1) at least *t* of the *Chlamydia trachomatis* antigens from either the first antigen group or the second antigen group, where *t* is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13, preferably *t* is five; (2) one or more antigens from another sexually transmitted disease. Preferably, the sexually transmitted disease is selected from the group consisting of herpes simplex virus, preferably HSV-1 and/or HSV-2; human papillomavirus; *N.gonorrhoeae*; *Treponema pallidum*; and *Haemophilus ducreyi.* These compositions can thus provide protection against the following sexually-transmitted diseases: chlamydia, genital herpes, genital warts, gonorrhoea, syphilis and chancroid (See, Ref. 101).

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity {*e.g.* refs. 102 to 111}. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM_{197} diphtheria toxoid is particularly preferred {112}. Other carrier polypeptides include the *N.meningitidis* outer membrane protein {113}, synthetic peptides {114, 115}, heat shock proteins {116, 117}, pertussis proteins {118, 119}, protein D from *H.influenzae* {120}, cytokines {121}, lymphokines, hormones, growth factors, toxin A or B from *C.difficile* {122}, iron-uptake proteins {123}, *etc.* Where a mixture comprises capsular

saccharides from both serogroups A and C, it may be preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Different saccharides can be conjugated to the same or different type of carrier protein. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

Toxic protein antigens may be detoxified where necessary *e.g.* detoxification of pertussis toxin by chemical and/or genetic means.

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used {*e.g.* refs. 124 to 132}. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein.

Definitions

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, *x*±10%.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 133. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in reference 134.

EXAMPLE 1: Immunizations with Combinations of the First Antigen Group

The five antigens of the first antigen group (OmpH-like protein, ArtJ, DnaK, CT398 and HrtA were prepared as described in reference 17. The antigens are expressed and purified. Compositions of antigen combinations are then prepared comprising five antigens per composition (and containing 15 μg of each antigen per composition).

CD1 mice are divided into seven groups (5-6 mice per group for groups 1 through 4; 3 to 4 mice for groups 5, 6 and 7), and immunized as follows:

Group	Immunizing Composition	Route of Delivery
1	Mixture of 5 antigens (15 μg/each) + CFA	Intra-peritoneal
2	Mixture of 5 antigens (15 μg/each) +AlOH (200μg)	Intra-peritoneal
3	Mixture of 5 antigens (15 μg/each) + AlOH (200μg) + CpG (10μg)	Intra-peritoneal
4	Complete Freunds Adjuvant (CFA)	Intra-peritoneal
5	Mixture of 5 antigens (5 μg/each) + LTK63 (5μg)	Intranasal
6	AlOH (200μg) + CpG (10μg)	Intra-peritoneal
7	LTK63 (5μg)	Intranasal

Mice are immunized at two week intervals. Two weeks after the last immunization, all mice are challenged by intravaginal infection with *Chlamydia trachomatis* serovar D.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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